THE UNIVERSITY *of* EDINBURGH

NEW DEVELOPMENTS IN FOOT-AND-MOUTH DISEASE AND THEIR POTENTIAL TO INFLUENCE FUTURE CONTROL IN BOLIVIA

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A dissertation submitted in partial fulfilment of

the requirements of the Master of Science Degree

in Tropical Veterinary Medicine

Centre for Tropical Veterinary Medicine

University of Edinburgh

1994

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ACKNOWLEDGEMENTS

The author wishes to thank his supervisor, Dr. G. R. Scott for his invaluable guidance and orientation during the course of this dissertation.

The author is also grateful to the staff of the CTVM for their tuition and advice during the year.

The author would like thank to Drs. J. McGrane, G. Mendez, D. Brown, S. Bishop and O.D.A. for their collaboration during this course.

The author is indebted also to his parents and brothers who provided constant love and support.

DEDICATION

with all my love to

LILIANA

for her self-sacrifice

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ABSTRACT

In the last few year scientists have advanced considerably our understanding of foot-and-mouth disease. This dissertation reviews these new findings and the potential for their application in a future FMD control programme in Bolivia is discussed.

Molecular biology studies have led to the development of specific diagnostic methods and have allowed identification of field isolates of the virus, techniques that immeasurably enhance the comprehension of the epidemiology of the infection and, therefore, allow more precise control measures to be implemented. Improvement in vaccines, however, has not been as fruitful.

Foot-and-mouth disease in Bolivia is one of the most important economic diseases of livestock and limits animal productivity. However, the disease in the country is uncontrolled and the national programme only covers small areas around the cities.

The different strategies for foot-and-mouth disease control are described and the possibilities for their applications in Bolivia are considered. The basis for a successful control programme in Bolivia should include non biological factors such as legislation and political decisions together with current technology. These strategies are undoubtedly tools for a successful programme of control. However it will only be possible if there is motivation and co-operation amongst the veterinarians, government institutions and livestock owners.

ABBREVIATIONS

A° = Atomic resolution

°C = Celsius degree

CF = Complement Fixation

CPFA = Centro Panamericano de Fiebre Aftosa

DTH = Delayed type hypersensitivity

DNA = Deoxyribonucleic acid

ELISA = Enzime-linked-immunosorbent-assay

FMD = Foot-and-mouth disease

FMDV = Foot-and-mouth disease virus

GIS = Geographic information system

ID = Infectious doses

INE = Instituto Nacional de Estadisticas

km = kilometres

LIDIVET = Laboratorio de Investigacion y Diagnostico Veterinario

Mabs = Monoclonal antibodies

MHC = Histocompatibility antigen complex

mRNA = Messenger ribonucleic acid

pH = Potential of hydrogen ions

RNA = Ribonucleic acid

RH = Relative humidity

RVF = Rift Valley fever

SVD = Swine vesicular disease

Th = T cell helper

Tc = T cell cytoxic

VE = Vesicular exanthema

VN = Virus neutralisation

VIIA = Virus associate antigen

VN = Virus neutralisation

VNA = Virus neutralisation antibody

VPg = Viral protein genome

VP1 = Virus protein 1

VP2 = Virus protein 2

VP3 = Virus protein 3

VP4 = Virus protein 4

VS = Vesicular stomatitis

WRL = World Reference Laboratory

1. INTRODUCTION.

Foot-and-mouth disease (FMD) is probably the most contagious disease which affects animals, especially the cloven-hoofed animals. The disease is characterized by an acute febrile condition with formation of vesicles around of the mouth, on the feet, on the teats and mammary gland. It is rarely fatal but muscular lesions in the myocardium are common in young animals and may lead to death.

Although the disease has a low level of mortality, in several countries FMD has remained a major scourge of livestock. Its high morbidity affects large numbers of animals and has disastrous effects on the national economy of any country. Likewise, FMD is one of the most important animal diseases because it spreads very fast through animal populations and if left uncontrolled inflicts very heavy production losses.

The severity of FMD varies widely in different regions because there are many strains involved and the immunity is not very well understood. Killed vaccines are available but have a low relative level of effectiveness, and they are difficult to administer in remote regions especially in developing countries.

Advances in biotechnology and molecular biology have permitted the development of new techniques for diagnosis and control. Enzyme-Linked-Immunosorbent-Assay (ELISA), cloning sequence, new biochemical tests, virus neutralization, computer simulation and other novel techniques will undoubtedly be

powerful tools for the control of the disease and understanding its epidemiology. Nevertheless the new generations of vaccines have not reached an adequate quality and still have many problems of immunogenicity, cost and security.

In South America movement of livestock, animal products and escape of FMD virus from laboratories and problems with vaccines appear to be the most important factors in the spread the disease in this continent. In Europe during the last two decades the major forms of FMD spread were escapes of FMD virus from laboratories, inadequate inactivation of FMD vaccines and air borne dissemination (Beck and Strohmaier, 1987).

The occurrence of FMD in Europe is decreasing and the disease is virtually eradicated by suspension of vaccination, strict movement control, and a stamping out policy. However in most of the South American countries FMD is endemic and the programmes are based on systematic vaccination. Della Porta (1983) commented that in South America nearly of 1000 million doses of FMD vaccine are produced in different laboratories; but in some of them there is not an adequate control of quality and number of vaccines produced is too high.

In Bolivia FMD is probably the disease that produces most economical losses in livestock. FMD is endemic and yearly there are many outbreaks in different parts of the country. National programmes of FMD control have managed only a low level of control near of the cities. Only voluntary vaccination is achieved by

farmers without adequate technical support. Hence the disease is practically uncontrolled in the country.

Therefore, the objectives of this dissertation are to review the new FMD techniques that have been developed and to discuss their potential for carrying out an FMD control programme in Bolivia.

2. BACKGROUND INFORMATION.

2.1. History of foot-and-mouth disease.

The earliest description of FMD disease was recorded in 1514 by Hyeronimus Fracastorius in northern Italy. There was confusion about the specific nature of the disease because of the difficulty of differentiating it from other contagious diseases, such as rinderpest and anthrax. Likewise FMD was recorded in Germany in 1751 and later in British dairies during July of 1836 (Anonymous, 1978).

Rosenberg and Goic (1973) commented that the first record of FMD in America was 1870. Simultaneous outbreaks were recorded in the United States of America (USA), Argentina, Chile, Uruguay and Brazil. In USA FMD was rapidly eradicated in 1929, the strategies to control the disease were: isolation, control of animal movement, quarantine, stamping out policy and slaughter. In South America there were no effective barriers to avoid the FMD spread at the end of 19th century and FMD was present in different countries in South America. In 1950 after importation of cattle from infected areas, FMD was reported in Colombia, Venezuela and Ecuador.

In Bolivia a very virulent outbreak was recorded in 1943. Reports from the Departments of Tarija and Santa Cruz indicated a very high FMD epidemic in livestock. Commercialization of cattle from productive areas permitted the dissemination of the disease and many outbreaks were recorded throughout the

country. This epidemic was attributed to careless inspection at the Bolivia-Argentine border and lack of inspection at Bolivian slaughterhouses (Machado, 1969).

In 1992 3 513 outbreaks of vesicular diseases in South America were recorded but only 1 190 were confirmed by the laboratory. The predominant type of FMD virus diagnosed was "O" with 576 episodes. FMD virus type "A" was identified 236 times and "C" 45 times. Moreover, Vesicular Stomatitis was diagnosed in 333 cases (Table 1). Chile, Guyana, French Guyana and Surinam continued to be free of the disease and Uruguay has not reported the disease since 1990 (CPFA, 1993).

In Bolivia FMD is endemic and in 1992, 18 cases were confirmed by the Bolivian Reference Laboratory. In 1993, 15 cases were diagnosed and in 1994 have been diagnosed 21 cases (LIDIVET, 1994). (Table 2).

Table 1. Distribution of vesicular diseases in South America - 1992.

Country	Clinic Reports	Samples collected	* FMDV type O	FMDV type A	FMDV type C	Vesicular Stomatitis
Argentina	350	293	108	72	39	0
Bolivia	228	35	18	0	0	0
Brazil	1 224	342	158	72	6	0
Colombia	1 308	957	226	82	0	309
Ecuador	174	46	30	0	0	0
Paraguay	43	32	23	0	0	0
Peru	94	68	12	3	0	13
Uruguay	0	0	0	0	0	0
Venezuela	92	32	1	7	0	11
Total	3 513	1805	576	236	45	333

* Foot-and-mouth disease virus.

Source: CPFA, 1993.

Table 2. Confirmatory diagnosis of FMD in Bolivia during 1990 - 1994.

Year	FMDV type O	FMDV type A	FMDV type C	Diagnosis not confirmed	Total
1990	9	5	0	8	22
1991	2	1	0	2	5
1992	18	0	0	17	35
1993	10	5	0	15	30
*1994	17	3	1	8	29
Total	56	14	1	50	121

Source: LIDIVET, 1994.

* 31/07/94

2.2. Geographic and socio-economic characteristics of Bolivia.

Bolivia is a land locked republic which lies between 10 and 22 degrees south of the equator in the center of South America (Figure 1). The country is divided ecologically into 3 different zones, the highland Andean plain, the Eastern valleys and the Eastern lowland plains which form the outlying reaches of the amazon basin, (Appendix 1)

Bolivia is divided geopolitically into nine departments. The human population is 6 525 000. The livestock population consists of 6 483 000 cattle, 4 617 000 sheep, 665 000 goats, 3 365 000 pigs, 352 000 equines, 785 000 South American Camelides and 7 452 000 poultry (I.N.E., 1993).

The mainstays of the Bolivian economy are the gas oil industry and mining which produce 61 percent the country's export earnings and these two activities contribute 55 percent of the GNP. However the agricultural sector provides employment for 68 percent of the country's work force although producing approximately only 35 percent of the GNP. Furthermore a substantial proportion of the agricultural work force are involved in subsistence farming, especially in remote areas of the Andean Plateau and the Amazon region (I.N.E., 1993).

Socio-economic importance of FMD in Bolivia.

FMD is a disease that has debilitating effects on livestock especially productive animals in endemic areas of the country.

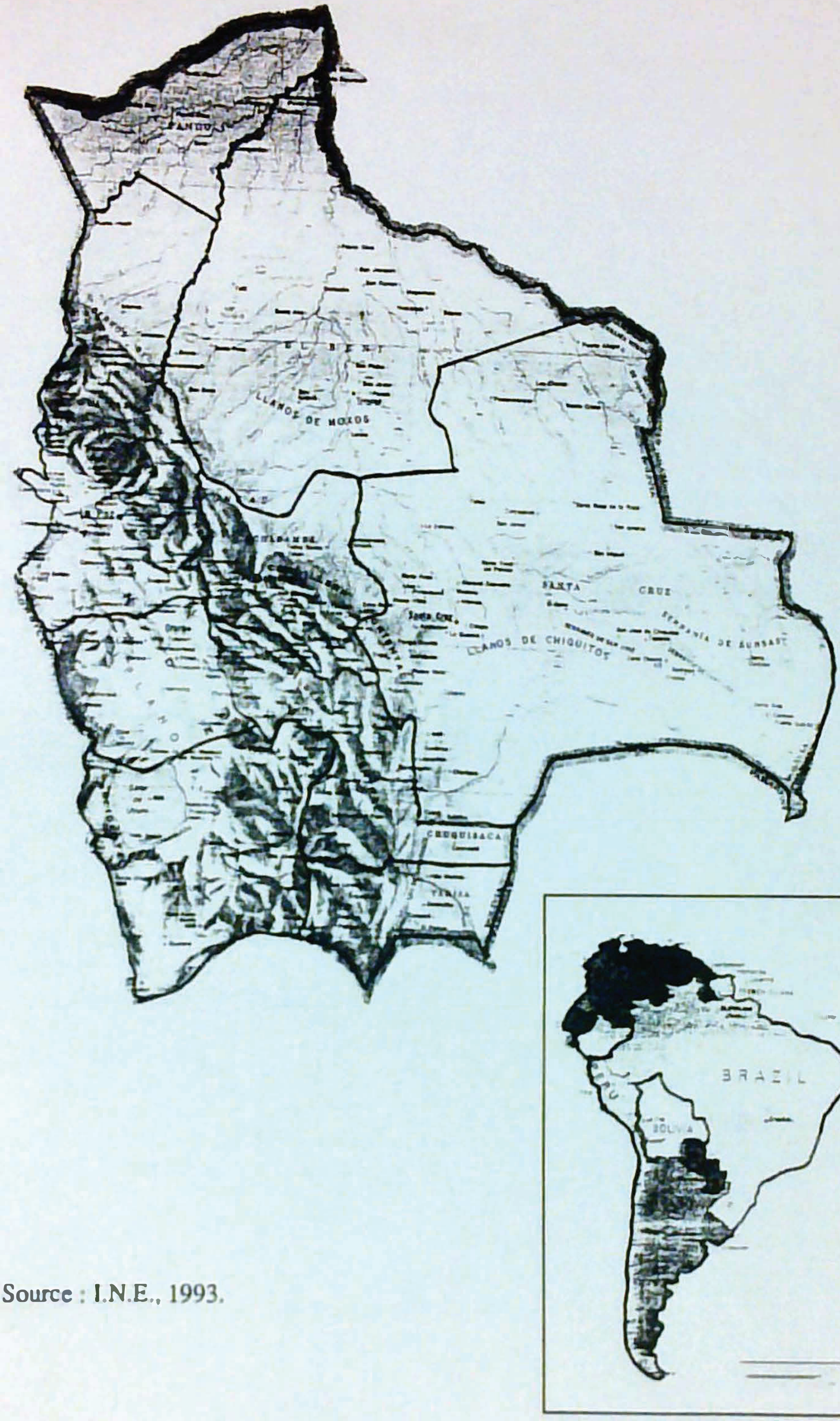
In Bolivia there is no evaluation of losses due to FMD. However in 1991 the C.R.P. (Agricultural Regional Council of the Department of Santa Cruz) considered that FMD produces losses of 80 000 000 US dollars yearly to the cattle industry in the department.

Likewise, in Bolivia FMD outbreaks are always present but many of them are not registered because the national programme of FMD does not have the resources to carry out surveillance and, at the moment, the disease is virtually uncontrolled (Tyler, 1991). Bolivia is a country in which approximately threequarters of the human population live in rural areas and cattle are the sole source of livelihood for many people. Consequently enormous losses due to FMD affect the regional and national economy.

Forman in 1990 considered that for developing countries in Africa, Asia, and South America FMD is significant in endemic areas because it causes the following constraints:

1. It reduces livestock productivity, including the draft capacity of animals used in the fields for crops
2. FMD excludes the opportunity for the exportation of animal products to FMD-free countries, which are often the most lucrative markets.
3. It limits the potential for upgrading livestock production using genetically superior animal.

Figure 1. Map of Bolivia.



Source : I.N.E., 1993.

3. AETIOLOGICAL DEVELOPMENTS.

The causal agent of foot-and-mouth disease (FMD) is an RNA virus that is a member of the aphtovirus genus of the picornaviridae family. It has a genome size of 8 300 nucleotides, the translation of which yields a single polyprotein. (Donaldson, 1987).

3.1. Classification.

The virus is classified into seven immunologically designated serotypes, viz., O, A, C, SAT-1, SAT-2, SAT-3 and ASIA-1. The O, A and C serotypes are prevalent in Africa, Asia, Europe and South America. The SAT-1 serotype occurs in Africa and Asia. The SAT-2 and SAT-3 serotypes are limited to Africa and West Asia. ASIA-1 occurs throughout Asia (Brooksby, 1968).

Pereira (1978) commented that in FMD there are biotypical strains that become adapted to particular animal species and infect other species only with difficulty; for example there are strains that are more virulent for pigs than for cattle and others less sensitive for guinea pigs than sheep. It has been observed that the antigenic structure of a virus appears to change during its adaptation to different species and the virus loses some of its components during the process.

A consequence of placing strains of FMD virus into groups or subtypes was the requirement to define the boundaries between subtypes (Brooksby 1982). Types were determined by serological techniques such as complement fixation (CF), virus

neutralization (VN) and, recently ELISA; other techniques such as cross-challenge experiments in cattle were used also (Roeder and Le Blanc Smith, 1987).

Within each type exists a number of strains, each designated a name related to its serotype, the area or country in which it was found and the year of its isolation. However as attempts to control FMD by vaccination evolved it was realized that antigenic differences existed between strains within each type. There was therefore a requirement to further classify the strains into antigenally similar groups or subtypes (Kitching, Knowles, Samuel and Donaldson, 1989).

Pfaff, Thiel, Strohmaier and Beck (1986) commented that there were a number of immunologically different subtypes with different degrees of virulence and the virus seemed to be capable of infinite mutation so that new, antigenically different subtypes were constantly appearing. Moreover there is no cross immunity between serotypes; immunity to one type does not confer protection against any of the other six types.

Kitching and his colleagues (1989) considered that a totally acceptable characterization method of nomenclature for FMD virus strains had yet to be formulated. As more nucleotide sequence data are accumulated, it may be possible to classify strains into closely related groups. By giving each of these groups a name or number a field virus could then be genetically classified. By combining this genomic classification (G number) with its antigenic classification (R number) an almost complete description of the strain would be possible. For instance, Utopia

3/88 R4 G6. Complete characterization of a strain in this manner enhanced the requirements of vaccine selection. In addition, geographic epidemic mapping of the strains could be elaborate for antigenic suitability, neutralization of the virus and for epidemiological purpose.

3.2. Molecular Biology.

Viruses are obligate intracellular parasites and, in nature, many of them give rise to latent infections causing little inconvenience to the host cells (Suback-Sharpe, 1968). However other virus host-systems caused profound changes in the host cell and frequently cause its death.

The picornavirus group induce inhibition of the host cell causing inhibition of the cell macromolecule synthesis. Presumably inhibition of cellular RNA and protein synthesis occur immediately after infection and this phenomenon is called "cut off" (Brooksby, 1982).

3.2.1. RNA amplification.

Montagnier, (1968) commented that the FMD virus-infection cycle is short, lasting only a few hours in the animal cell and ending in lysis of the infected cell releasing the virion into the incubation medium. The replication of the FMD virus occurs after adsorption, penetration, and uncoating, VPg (viral protein genome) is removed from the virion RNA by cellular enzymes. The virion RNA, acting as mRNA, is translated without interruption into a polyprotein. This is then cleaved into four primary products, which are further cleaved into smaller proteins. One of

the primary cleavage products is cleaved into the four structural proteins, and another is the precursor of viral moiety of RNA polymerase. The function of the other two primary polypeptides is unknown. The RNA polymerase transcribes a complementary negative (-) sense strand that in turn serves as a template for the synthesis of new positive (+) sense strands. These positive sense strands then act as additional mRNAs for the further synthesis of viral protein or are incorporated into progeny virions (Rueckert, 1985).

Picornaviruses are unique among the RNA viruses in that when a cell is doubly infected with two indistinguishable strains of the same species of virus, the virus genomes may undergo intermolecular recombination, a feature demonstrated with both foot-and-mouth disease virus and polio-virus (Rueckert, 1985).

3.3. Capsid proteins.

Sangar and Clark (1986) considered initial cleavages of polyprotein by host cell protease resulted in four primary products. Further cleavages by virus-specified protease produced the final virus-induced four major structural polypeptides or capsid proteins.

Kitching and his collaborators in 1989 showed that the intact FMD virus particle is made up of another capsid consisting of 60 copies of the four capsid proteins, 1A, 1B, 1C, and 1D, (previous nomenclature virus protein (VP) VP4, VP2, VP3 and VP1) enclosing one molecule of single-stranded RNA. The sedimentation constant of the particle is 146S. Empty FMD virus particles have a

sedimentation constant of 75S and are structurally similar to the intact particle but contain no RNA and the two proteins 1A and 1B remain covalently linked as 1AB. FMD infectious particles have a sedimentation constant of 12S and are pentamers of the protein 1B, 1C and 1D: produced by suspending the intact particle in acid media or by heating.

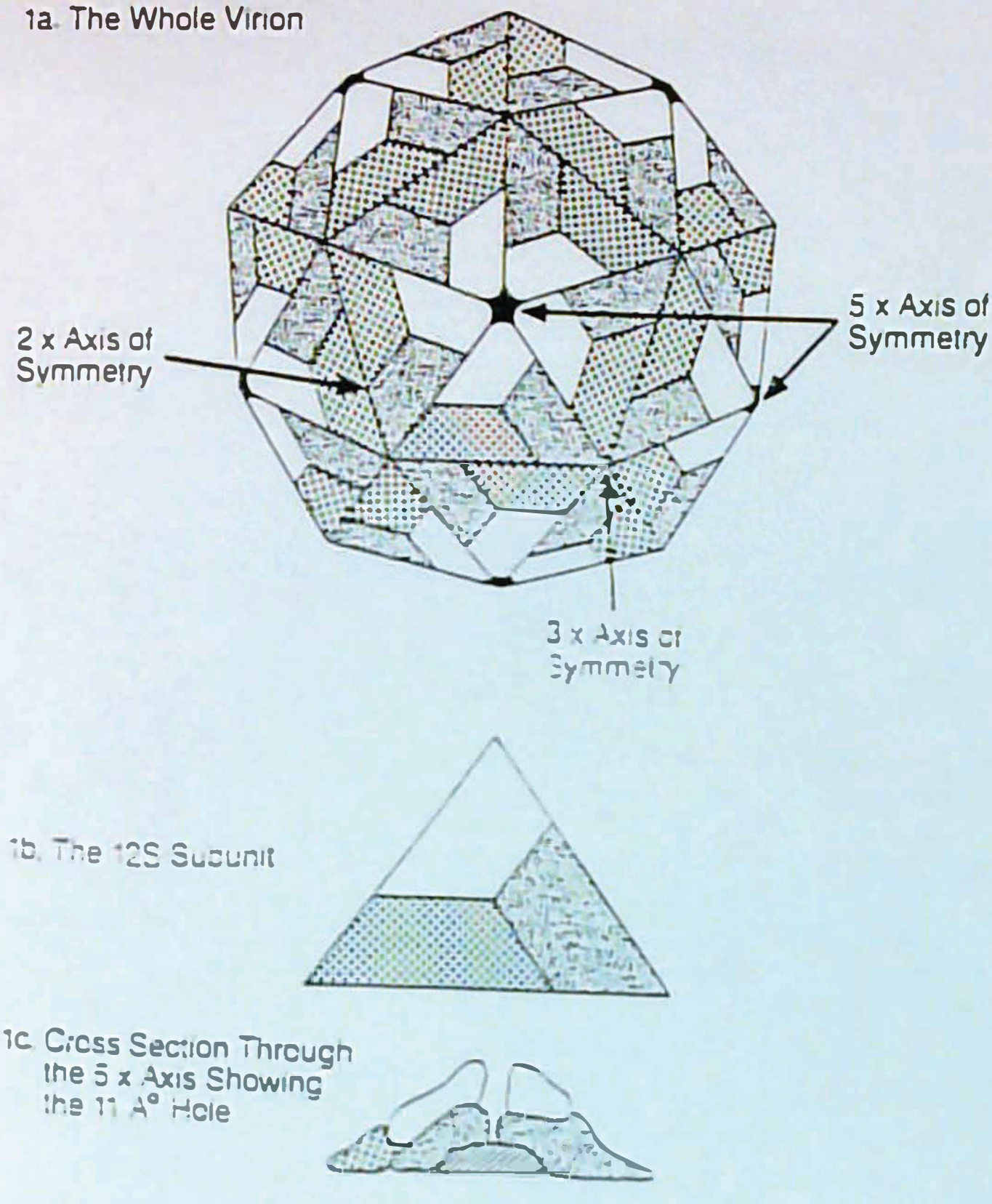
Burroughs, Rowlands, Sangar, Talbot and Brown (1971) considered that capsid proteins are coded by 5' end of the RNA. In comparisons between nucleotide sequences of RNA purified from FMD viruses of the seven different serotypes, the antigenic differences between the seven serotypes reside in differences in the external configuration of the virions, which is determined by the structure of capsid proteins. The study of antigenic sites on the surface of FMD virus has been facilitated by the development of techniques to produce antibodies which react with individual antigens sites i.e. the so called monoclonal antibodies (Kitching et al, 1989).

Rowlands, Sangar and Brown, (1971) showed that two distinct immunogenic sites are present on the surface of FMD virus. One is concerned with the adsorption of the virus to susceptible cell as well as the production of neutralizing antibody. The re-movement of this site with trypsin did not affect the gross morphology of the virus but the particles then had a reduced infectivity. The second immunogenic site in trypsin-treated particles also produce neutralizing antibody. In addition, Strohmaier, Franze and Adam (1982) considered that the structures formed by amino acid sequence's 146-154 and 201-213 of 1D defined the

main immunogenic site of FMD virus. Likewise, Rowlands, Clarke, Carroll, Brown, Nicholson, Bittle, Houghten and Lerner (1983) observed that 141-160 of capsid polypeptide VP1(1D) contained the major immunogenic site of the virus. These results have practical implications for the choice of viruses for vaccine production.

Acharya, Fry, Stuart, Fox, Rowlands and Brown (1989) showed the tridimentional structure of FMD virus at 2.9 A^{o} resolution (Atomic resolution by X-ray diffraction); differentiates the different proteins and antigenic sites on the surface of the virus (Ouldridge, 1990). (Figure 2.)

Figure 2. Diagrammatic representation of the structure of foot-and-mouth disease virion and capsid proteins.



Source: Ouldridge (1990).

4. DIAGNOSTICAL DEVELOPMENTS.

Hamblin, Barnett and Hedger (1986) explained that foot-and-mouth disease is usually, initially diagnosed clinically and confirmed by virus isolation and identification. Clinical diagnosis is relatively easy in cattle and pigs in an area where existing outbreaks have been recognized. It is however, very difficult to diagnose clinically in sheep.

4.1. Presumptive diagnosis.

Aphthovirus infects a wide variety of cloven-hoofed domestic and wild animal species (Brooksby, 1972). In South America most clinical cases are observed in cattle, pig, sheep and occasionally South American Camelids (Lubroth, and Yedloutschnig, 1987).

Cattle. After an incubation period of 2 to 8 days, Infected cattle exhibit anorexia, diminution in milk production and rise in temperature 40-41 °C (Fig. 3). Within 24 hours vesicles develop in upper surface of the tongue, dental pad, lips, and muzzle. At the first vesicles appear as whitish blister of 1-2 cm of diameter, but these rapidly increase in size and may cover large areas of buccal mucosal and tongue producing salivation and buccal epithelial lesions (Fig. 4). Vesicles also can be found in the inter digital skin, coronary band of the feet and on the bulbs of the heel. In sever attacks the claws may shed (Sard, 1978).

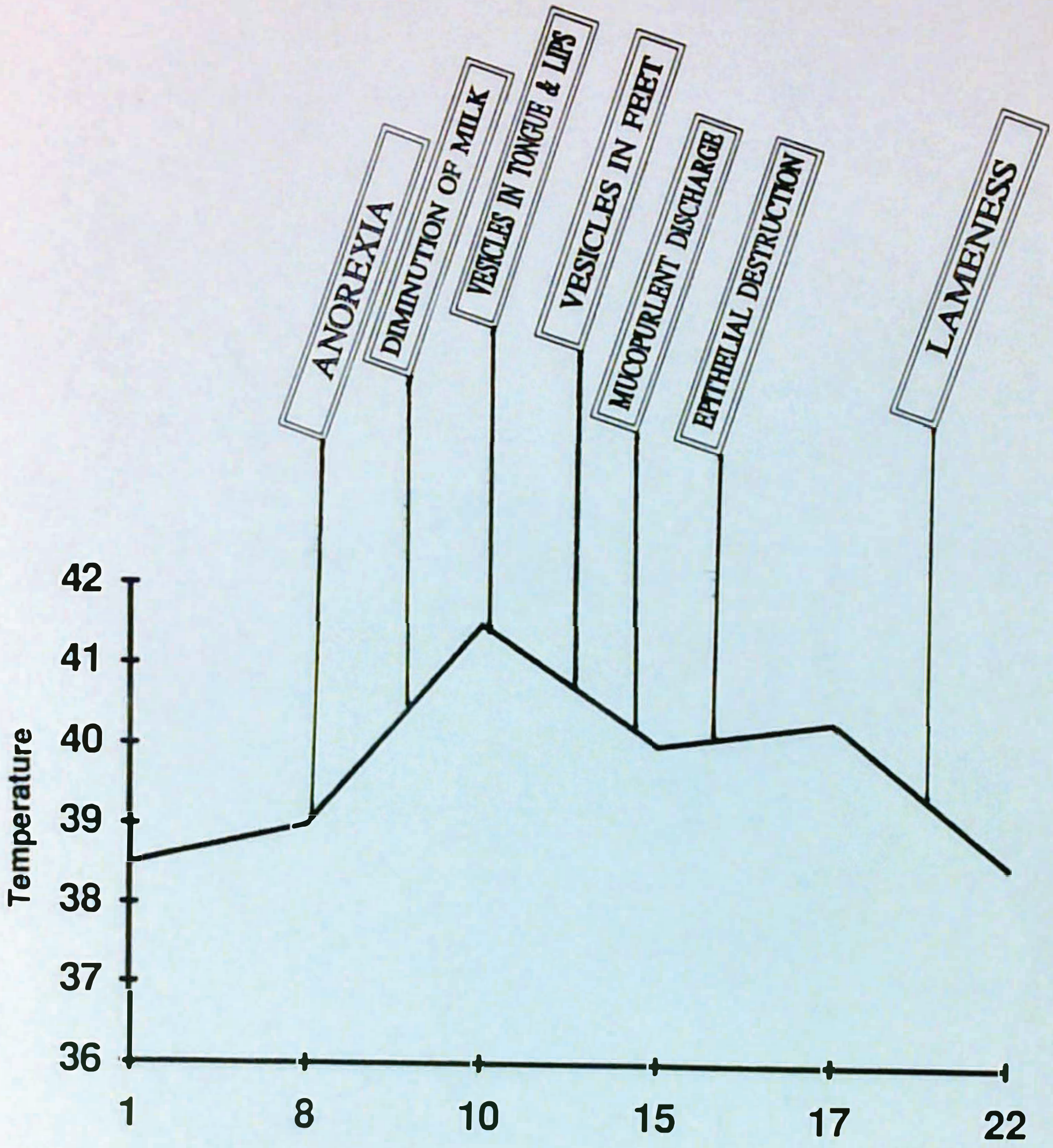
Pain and fever are most intense during formation of the oral vesicles and subside after their rupture. An intense stomatitis with severe epithelial destruction of buccal muccosal and tongue (Fig. 5) leads to a copious production of saliva which froths round the lips and nose and drools from the mouth. In non-lactating animals drooling saliva is often the first observed sign.

Lesions on the tongue often heal within 2 or 3 days but on the feet and within nasal cavities often become secondarily infected with bacteria, resulting in lameness and mucopurlent nasal discharge.

Other painful vesicle lesions may develop on the udder and teats of cows and heifers that resent being milked, producing permanent damage in the udder and a lesion that permits bacterial invasion and severe secondary mastitis (Brooksby, 1972).

Mortality in adult cattle is very low. Although the virus does not cross the placenta cows may abort due to high temperature. In calves up of 6 months of age, FMD virus can cause death through myocarditis (Burrows, Mann, Garland, Greig and Goodridge, 1981).

Figure 3. Relation of temperature and lesions of FMD in cattle



Source: Sard, 1978.

Figure 4. Salivation and buccal lesions in cattle.

Source: LIDIVET, 1994

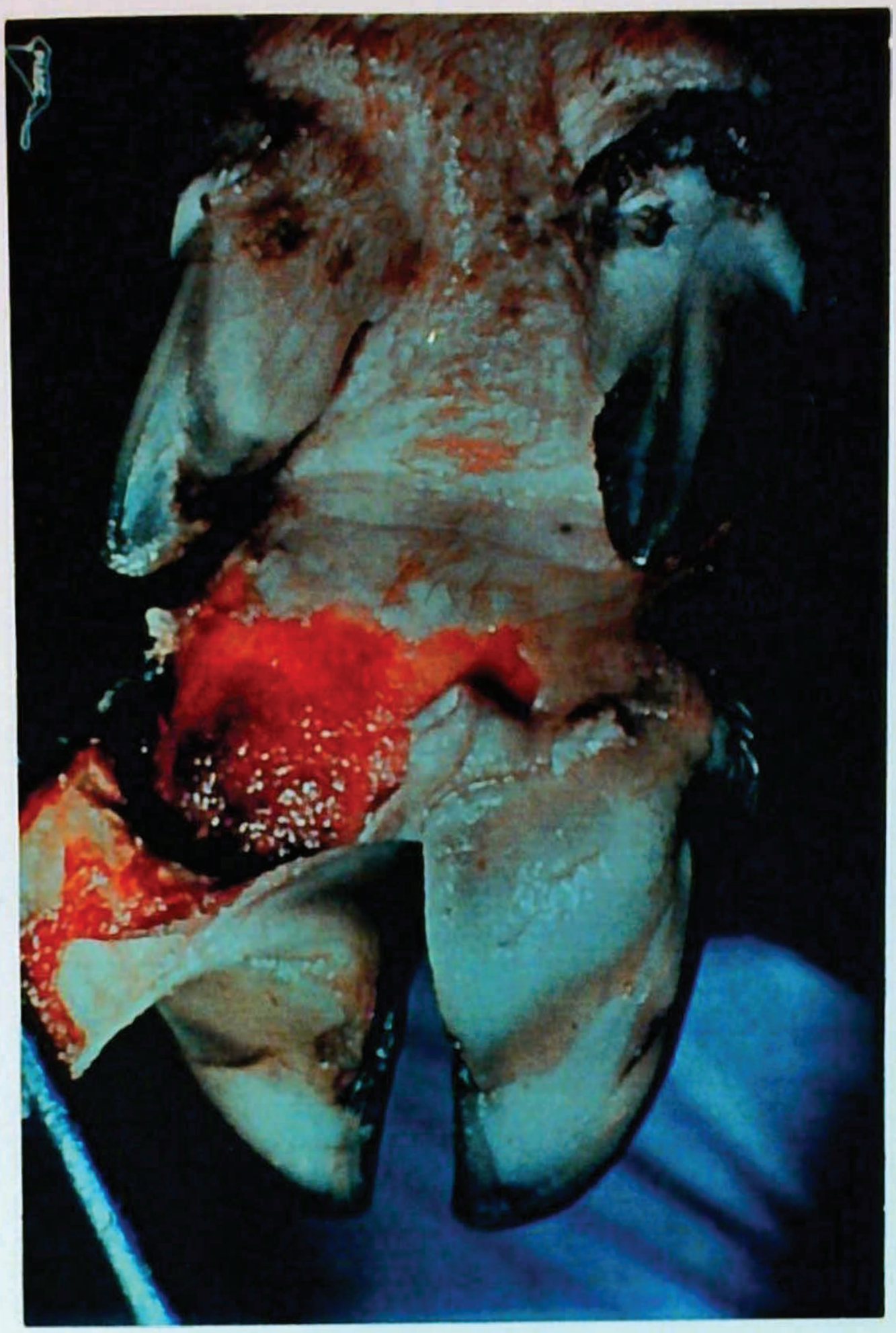
Figure 5. Epithelial destruction of tongue in cattle.

Source: LIDIVET, 1994.

Swine. The incubation period of FMD in pigs is longer than that in cattle, viz., 6 to 12 days. Fever and lameness are often the initial symptoms. Foot lesions may be severe and may be sufficiently painful to prevent pigs from standing. Denuded areas between the claws and supernumerary digits usually become infected with bacteria causing suppuration and, in some cases, loss of the claw prolonging lameness (Fig. 6.). Vesicles within the mouth are usually less prominent than in the cattle, although large vesicles, which quickly rupture, often develop on the snout (Terpstra, 1972).

Death without premonitory signs and associated with myocarditis is a well-established feature of foot-and-mouth disease in young animals. Recently attention has been draw to sudden deaths of new-born piglets from FMD in which piglets show myocarditis without developing vesicles (Donaldson, Ferris and Wells, 1984).

Figure 6. Lesion of the feet in swine.



Source: LIDIVET, 1994.

Sheep. In sheep acute lameness of sudden onset is suggestive of foot-and-mouth disease. Usually, but not always, all four feet are affected. The pain is intense painful so that the animals are reluctant to rise or move, adopt a crouching position with the hind legs brought forward. Vesicles may extend all round inter digital cleft, and they may cause separation of the hoof from underlying tissues. Although rupture of vesicles may cause dampness of hair round of the hoof, the area is clean and lack of "foot rot" odor. Oral lesions are seldom noticeable in sheep but vesicles are occasionally found on the tongue and dental pad (Sard, 1978).

South American camelids. Lubroth and Yedloutschnig (1987) reported that alpacas, llamas and vicunas are susceptible to FMD virus and clinical disease is observed with formation of vesicles in the feet and occasionally in the mouth but the outbreaks in South America of FMD in llamas, alpacas and vicunas are rarely documented. Furthermore Lubroth, Yedloutschnig, Culhane, and Mikiciuk (1990) reported that llamas after 5 to 7 days exposure to FMD virus, generally developed clinical disease with pyrexia, some vesicular lesions on all four extremities but not in the mouth. Confirmatory diagnosis by virus neutralization (VN) test and agar immunodifucion test for virus associated antigen (VIAA) revealed antibodies to FMD.

Other animals. The clinical disease in the goat, and wild ruminants is usually milder than in cattle and is characterized by feet lesions accompanied by lameness.

4.2. Differential diagnosis.

Three other viruses, together with FMD virus, affect animals with the formation of vesicles and they are clinically indistinguishable. These are: vesicular stomatitis (VS), vesicular exanthema (VE) and swine vesicular disease(SVD). Prompt and accurate diagnosis of these diseases therefore is essential (Cottral, 1969).

Vesicular stomatitis.

A common infection of cattle in the Americas is VS which is clinically similar to FMD. The morbidity of vesicular stomatitis in sheep and goats is much lower and more sporadic than FMD and FMD does not occur in horses. In VS the transmission of the disease will be by *Phlebotomus spp* (sand fly) and the mosquitoes *Aedes aegipty*. Differentiation on clinical and epizootiological grounds between FMD and VS could be dangerous (Hanson, 1981).

Vesicular Exanthema.

The natural hosts of VE virus are marine mammals. Spread to pigs occurs when the pigs are allowed to walk freely on seashores in which colonies of seal or sea lions are living.

The disease is indistinguishable clinically from FMD, VS and SVD. VE has a lower morbidity than FMD and only has been reported in USA (Madin, 1981).

Swine Vesicular Disease.

SVD, apparently is a new disease probably acquired from man that is spreading rapidly throughout the world. This disease is indistinguishable from FMD, affects swine and under experimental condition sheep were to have subclinical infections. The occurrence of SVD is associate with lesions resulting from traumas to the feed (Mann, 1981).

4.3. Confirmatory diagnosis.

Complement Fixation (CF).

The CF test for antigen or antibody is based on fixation of complement by antigen-antibody aggregates and the method for FMD is described by Casey (1965).

For many years, the direct complement fixation (CF) test was routinely used in the World Reference Laboratory (WRL) in Pirbright and in other diagnostic laboratories in the world for detection and typing of FMD viruses in epithelial samples from the field. No problems are experienced in the testing of samples containing sufficient epithelium from fresh, recently ruptured vesicles. However inadequate amounts or poor quality of the samples gave inconclusive or anti-complementary result (Hamblin, Armstrong and Hedger 1984).

Virus neutralization (VN).

The test has been used for many years to measure antibodies against FMD viruses and VN titres recorded after vaccination in cattle and mice. However in the recent years ELISA, used for detection of antibodies of FMD virus, is considered to be more precise than the VN test and is more reliable for the measurement of

antibody status of infected and vaccinated animals. Moreover, ELISA can measure antibodies which appear relevant to protection but which are not measured in VN test. This is important in the differentiation between vaccinated and infected animals (Hamblin, Barnett and Crowther, 1986).

Virus isolation in animals

Primary recoveries of field strains are made in primary goat, bovine and hamster kidney cell cultures. Recovered virus is identified by pathogenicity test in these animals.

Young adult guinea pigs are inoculated intradermally into the foot and vesicular lesions are observed in 24 to 48 hours. The virus can be demonstrated in blood at the time of the first initial lesions. The disease is usually not fatal in guinea pigs. Suckling mice (7-14 days old) inoculated intracerebrally develop spastic muscular paralysis in 1 to 2 days later (Warren, 1969).

ELISA (Enzyme-Linked-Immunosorbent-Assay).

In the last few years, in the World Reference Laboratory (WRL) for FMD diagnostic at Pirbright in the United Kingdom (UK), the Enzyme-Linked-Immunosorbent-Assay (ELISA) has replaced the complement fixation (CF) and virus neutralization (VN) for FMD antigen detection, serology test and for the detection and serotype identification of the virus. (Hamblin, Armstrong and Hedger, 1984).

The double-antibody ELISA is used for evaluation, detection and serotype identification of FMD antigen in epithelial tissue. This test has been found to be more sensitive and specific than the CF test (Roeder and Le Blanc Smith, 1987). Furthermore, Oliver, Donaldson, Gibson, Roeder, Le Blanc Smith and Hamblin (1988) confirmed that ELISA for detection of antigen in bovine epithelial samples is more specific and sensitive than CF and also indicates both the site of origin of the epithelial tissue sample (foot or mouth) and age of the lesion from which the sample is collected. These are important factors in influencing laboratory diagnosis of FMD. In cattle, foot lesions are more likely to yield antigen than mouth lesions and to remain positive for longer period.

Hamblin, Armstrong and Hedger (1984) considered that ELISA requires only one species of antiserum and used smaller volumes of test sample and reagents. Furthermore, Crowther (1986) considered that the versatility of solid phase microplate ELISA has been fully exploited in the study of problems associated with FMD. Three areas of most importance are:

-FMD virus typing

-FMD antibody detection

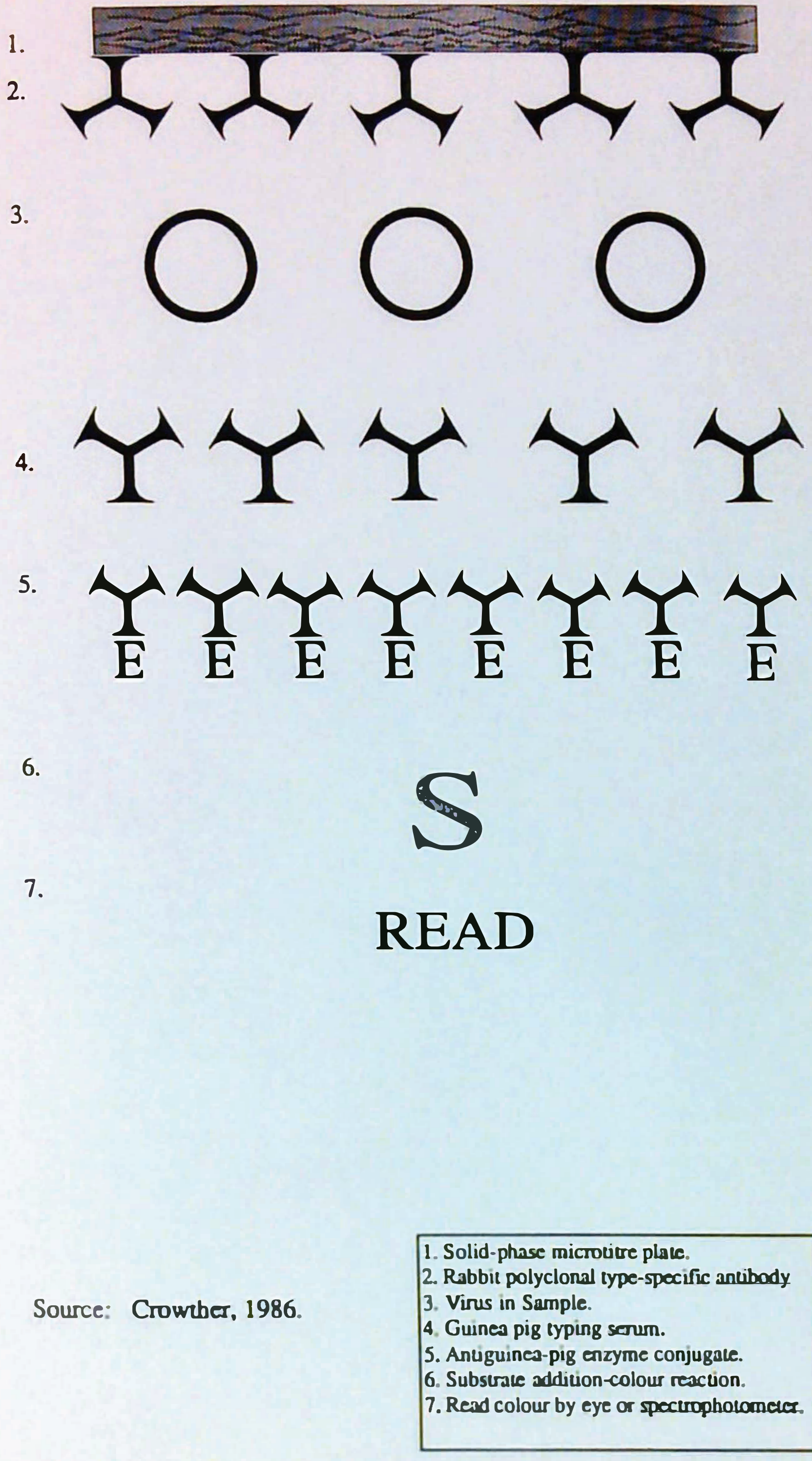
-FMD virus subtyping

The majority of these procedures involve the use of monoclonal antibodies adsorbed to the plastic microtitre plates to trap virus antigen possibly contained in a sample, and then a second antiserum from a different species is used to type and detecting the trapped antigen. After full validation of ELISA in the WRL for FMD

enough reagent was produced in Pirbright to supply the world typing need for several centuries. The basis of the assay is show in the figure 7.

In addition, Ferris and Dawson, 1988 determined that depending on the geographical origin of the samples it may appropriate to simultaneously ELISA for FMD, vesicular stomatitis and for swine vesicular disease.

Figure 7. Stages of ELISA for serotyping of foot-and-mouth disease.



Source: Crowther, 1986.

1. Solid-phase microtitre plate.
2. Rabbit polyclonal type-specific antibody
3. Virus in Sample.
4. Guinea pig typing serum.
5. Antiguinea-pig enzyme conjugate.
6. Substrate addition-colour reaction.
7. Read colour by eye or spectrophotometer.

Monoclonal antibodies (Mabs) for FMD viruses

Mabs are antibodies produced artificially from a primed cell clone and therefore consist of a single type of immunoglobulin. Monoclonal antibodies are produced by fusing antibody-forming lymphocytes from mouse spleen with mouse myeloma cells, the resulting hybrid cell multiplies rapidly as a cancer cell and produces the same antibody as the parent lymphocyte (Fazekas de St. Groth and Scheidegger, 1980).

Monoclonal antibodies for FMD virus have been studied mainly for research interest to investigate for example the location and relationship of important epitopes, the mechanism of virus neutralization and amino-acid of Mabs-induced mutants (Kitching et al, 1989).

The ELISA has been used extensively to measure binding of Mabs to various antigenic preparations both in the initial screening of antibody and for characterization of reactivity of Mabs. It is clear that viruses may be compared according to their ability to bind with Mabs in a relatively straight forward ELISA and that binding can be correlated with biological properties of the virus such as the position of neutralizing antibody and epitopes.

It is therefore very relevant to cross-protection between vaccines and field strains (Crowther 1986).

Roeder and Le Blanc Smith (1987) commented that some laboratories have produced hetero-hybromas by the fusion of bovine murine myeloma cells, so that cells secreting bovine antibody against FMD have been cloned. Such antibodies are prepared from bovine material (post-infection or vaccinated animals) and therefore the antibodies isolated have a direct bearing in the epitope recognized by cattle in the field, as compared to antigenic recognition of murine systems.

5. VACCINE DEVELOPMENTS.

Vaccination is the main attempted method of control for FMD disease in many countries. However the use of formalin-inactivated commercial vaccines has been the origin of many outbreaks in many parts of the world (Barteling and Vreeswijk, 1991). In 1987 Beck and Strohmaier found that of 18 strains isolated from different outbreaks of FMD in Germany, 14 were induced by a few types which had existed endemically in Europe for more than 20 years and were used for the production of vaccines. Furthermore, they considered that the many of the outbreaks in Europe during the eighties were "home-made" and not introduced from outside. Many of them probably were induced by vaccines, especially formalin-inactivated or escapes from vaccine production plants.

5.1. Current vaccines.

Brown (1987) commented that vaccines which are used currently are prepared by growing the virus in a fragment of tongue epithelium or in baby hamster kidney cells and then inactivating it with an agent such as acethyleneimine. Over the years, strains of virus which grew to higher titres in appropriate tissue culture systems have been isolated and, today more than one billion doses of vaccines are produced routinely from many laboratories of the world.

Constraints of vaccination.

In many countries where FMD is endemic the extensive use of killed FMD virus vaccines is a common practice for prophylaxis and control. However the

vaccines have many constraints such as antigenic variation of different serotypes and subtypes, and changes in the virulence of the virus. The immunity conferred by the vaccines against one serotype leave the animal susceptible to infection by the other six (Rowlands, Clarke, Carroll, Brown, Nicholson, Bittle, Houghten and Lerner, 1983) and even to other subtypes within the serotype (Salt, 1993). In addition Brown (1987) numbered the following disadvantages of FMD-killed vaccines :

1. Their limited shelf-life, making it essential to store them at refrigerator temperatures.
2. The need to use a cold chain, to ensure that they reach the animal in good condition .
3. The need to have facilities for the production of very large amounts of virus under conditions of high security to prevent its escape to the environment.
4. The need to ensure the complete inactivation of the virus.
5. The occasional occurrence of delayed type hypersensitivity (DTH) reaction.

✓ Donaldson and Kitching in 1989 commented that vaccinated cattle that have had contact with live FMD virus continue to carry virus in their pharynx. Over 60 per cent of these cattle carry virus for periods longer than two months, following contact, in spite of having high levels of neutralizing antibody, suggesting that the carrier state is the normal consequence of the infection. Immunological investigations into the mechanism of persistence has shown that carriers animals,

rather than having an impaired immunological response to FMD virus, have higher levels of immunoglobulin A (IgA) in their pharyngeal secretion than non-carriers. The carrier state may thus be of mutual benefit to the bovine, and to the virus which persists, and under as yet undefined circumstances, initiates new outbreaks of disease.

In South America nearly to 1 300 million doses of FMD vaccine are administered annually and vaccination has been widely used particularly in meat-exporting countries (Della Porta, 1983) with the inherent disadvantages of thermal instability, virus serotype specificity and short term duration of protection (Parkhouse, 1994). However in South America FMD control has been based on prophylaxis, despite the absence of a solid veterinary infrastructure. Polyvalent vaccination carried out at four or six monthly intervals on the largest cattle population attainable, was aimed at creating a biological barrier to subtypes and strains distributed over immense territories, most of them open to the infection especially in the core of the continent.

Taking into account the conditions under which animals are kept, handled and immunized in South America, as opposed to Europe, and to the fact that in many areas the entire sheep population is not included in the vaccination programme, it would be unrealistic to expect substantial territorial gains in disease control by vaccination (Boldrini, 1978.)

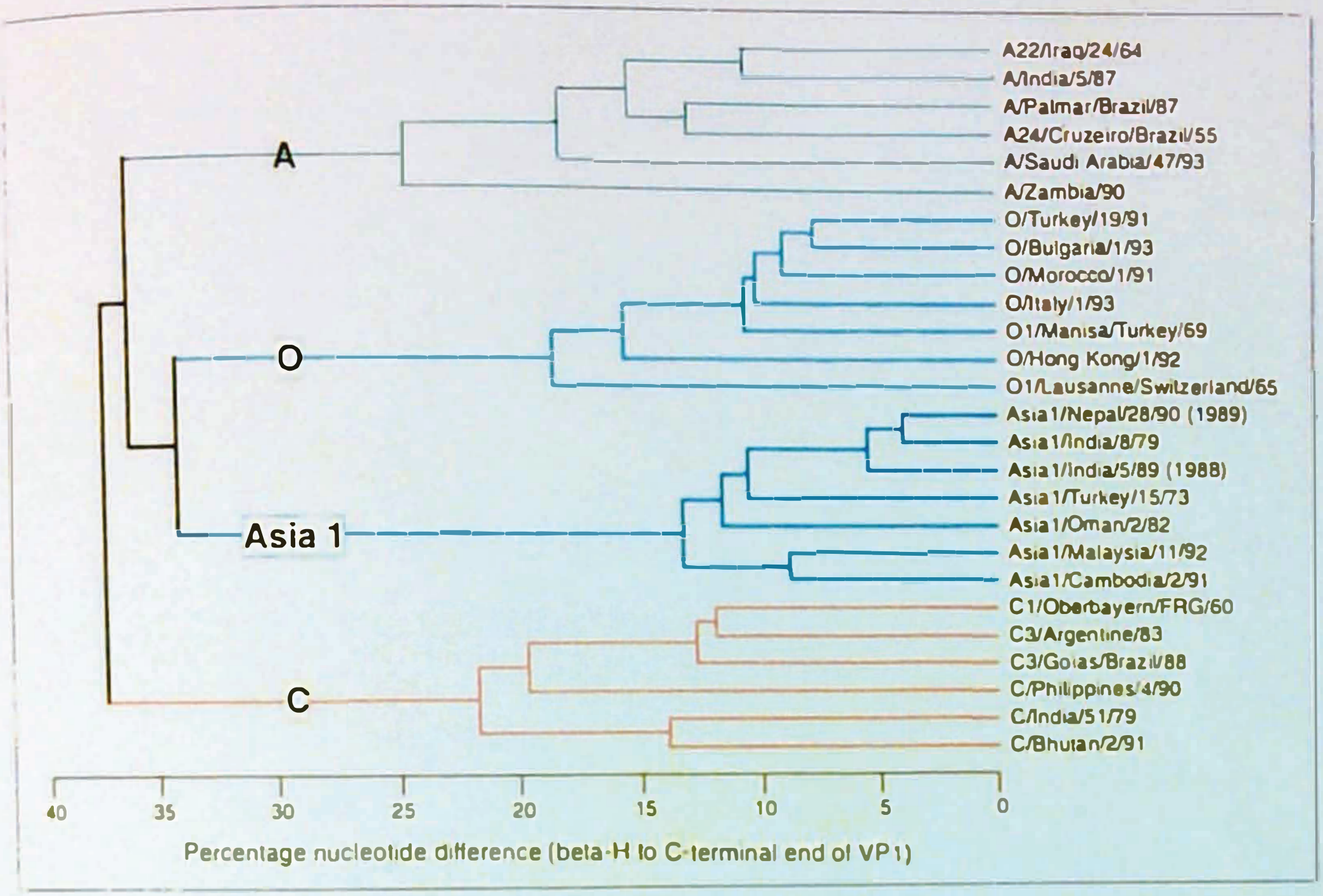
Nevertheless in South America, Uruguay using vaccination and control of animal movement controlled clinical FMD (CPFA, 1993)

Nucleotide sequence and vaccines.

Kitching (1992) commented that another valuable tool for studying the persistence and transmission of FMD virus is the nucleotide sequence of viral genome. Using this technique it is possible compare the evolution of FMD strains because, every FMD serotype and even subtype gives a very distinct sequence. This sequence is called the "fingerprint" of the virus, (Kurz, Forss, Kupper, Strohmaier and Schaller, 1981) and it has been used in the conformation of FMD virus strains maps.

Likewise Salt (1994) explained that Pirbright (UK) had established an International Vaccine Bank where by nucleotide sequence strains are held in a portfolio of antigens of current FMD virus strains. This portfolio has been used in the manufacturing of oil vaccines. A diagram of FMD nucleotide sequence strains is shown in figure 8.

Figure 8. Nucleotide Sequences of FMDV strains in the International Vaccine Bank at Pirbright UK.



Source : Salt, 1994.

5.2. New Vaccines.

Immunogenic considerations.

Ada (1990) pointed out that vaccination was generally used as a form of immuno-prophylaxis, so that administration of the vaccine even a long time prior to exposure to the wild type virus organism should afford some protection. Since T and B cells of the blood are short-lived, *viz.* only for few days, a prime requisite of a vaccine is to generate an immunologic memory as follows:

1. Activation of antigen presenting cells to initiate antigen processing and production of interleukins.
2. Generation of T helper (Th) and T cytoxic (Tc) cells to several epitopes to overcome the variation in the immune response in the the population due to major histocompatibility antigen complex (MHC) polymorphism.
3. Persistence of antigen, probably to dendrictic follicular cells in lymphoid tissue, where B memory cells are recruited to form antibody-secreting cells that will continue to produce antibody.

Although FMD-immunity is partially due to neutralizing antibody, it is poorly understood. Nonetheless important are cell-mediated components which provide an essential regulatory role in the induction and expression of the neutralizing response. Previous work has established that a synthetic peptide of FMD virus comprising the two immune-dominant VP1 epitopes (residues 200-213 and 141-158) partially protect cattle. This variable stimulation of protective

immunity presumably correlates with the observed MHC - linked T cell recognition of the peptide. (Parkhouse, 1994)

Synthetic vaccines

Brown (1987) explained that over the last 30 years the parallel development of molecular biology and the capability of studying structure function relationships at the molecular level, accumulated knowledge on structural features of foot-and-mouth disease virus which are involved in eliciting the immune response.

Four virus-specified particles have been identified in virus harvests produced in tissue culture cells. The particles, which were identified by their reaction with the serum from animals which had been identified with virus are the infectious particles sedimenting at 146S, 75S, 12S and 4S, which have a relative immunogecity of 100 per cent, 1 per cent, 1 percent and 0 per cent respectively. Moreover it is considered that the important factor which emerged from these studies was that intact virus was by far, the most immunogenic form. Empty particles and the 12S subunity also induced the formation of neutralizing antibody but during studies to understand the immunogenicity of the complete virus the low activity of the other particles became apparent.

Doel, Gale, Do Amaral, Mulcahy and Dimarchi (1990) explained that a number of laboratories have attempted to develop alternative vaccines based on either the viral coat protein, VP1, or synthetic peptides specificity recognizing the same proteins and they considered that 146-160 region of VP1 was clearly identified as the major site for infection of virus-neutralization antibody (VNA).

Parry, Ouldridge, Barnett, Clarke, Francis, Fox, Rowlands and Brown (1989) considered the major design objective for peptide vaccines was maximal immunogenicity allied to broad cross-reactivity. Peptides extended by natural continuous sequences at the amino acid terminus beyond amino acid 141 -160 have increased immunogenicity, but the comparison of antisera to peptides 141-160 C and 135 -160 indicated that the specificity also increased. Parry, Fox, Rowlands, Brown, Fry, Acharya, Logan and Stuart (1990) noted that the short FMD virus peptide evoked a more cross-reactive antibody response and Parry and collaborators (1989) considered that 145-150 region constituted a major component of a Mab which neutralized a range of type O strains. Furthermore it became clear that relatively minor manipulation of native sequences or single amino acid substitution resulted in increases in both the homotypic and the heterotypic reactivity of antisera raised against FMD virus peptide.

The major problem remaining in the development of synthetic peptide vaccine against FMD virus is that none, so far, elicit a high-titre antibody response in cattle, but the identification of appropriate cattle T cell epitope which can be mimicked by short linear sequences may be one of the last important steps in the provision of a successful synthetic vaccine .

Doel (1991) emphasized that synthetic peptides for FMD vaccines did not suffer problems of residual infectivity and peptides A40, C40 and O40 could confer significant levels of homotypic and heterotypic protection, but the sequences responsible for individual hetereotypic protection were unknown, although the R-G-

D sequence within the 141-158 region of VP1 had been implicated. Furthermore in peptide vaccines there are both positive and negative aspects; on the one hand, the consequence of inducing antibody to a sequence which binds to a protein on the surface of a cell needs to be considered. It is possible that inadvertently induced antibodies will inhibit the natural role of FMD receptor protein on susceptible cell. On the other hand, it is intriguing that it was possible to provoke antibody to a hetereotypic determinant in the virus which was otherwise silent even with repeated doses of conventional FMD vaccines This suggests that the use of carefully tailored synthetic antigen will allow the stimulation of useful immune responses where either antigen variability or lack of immunogenicity of a whole pathogen makes the induction of a protective immune-response difficult or impossible.

In addition, a number of other approaches should be examined for their ability to induce qualitative superior immune responses. For example, the route of administration and the structure of peptides in relation to T cell epitopes may influence greatly the immune response of the target species. With regard to T-cell epitopes, the incorporation of potent sequences may effect more relevant isotype switching and memory induction and work is in progress to examine this question (Doel et al, 1990). The route and mode of administration, may target the peptides towards more appropriate antigen presenting cells and stimulate more effective immune responses through the incorporation of novel adjuvants. Perhaps the most attractive, economic and practical approach especially in developing countries, is to deliver peptides by means of an attenuated viral vector acceptable for use in farm

animals. Given the range of options, the prospects for commercial synthetic FMD vaccines remain good.(Doel, 1991)

Other Vaccines.

From the time of Pasteur, virus strains suitable for live virus vaccines were modified chiefly by selecting naturally attenuated virus or by cultivating the virus serially in various foreign host or cells in the hope of attenuating it. The research for such strains is now being approached by laboratory manipulation to create specific alteration. (Melnick, 1985).

Hubbard, Baskerville and Stephenson (1991) showed that a live attenuated vaccine variant of Rift Valley fever (RVF) virus was developed by passing a human isolated in tissue culture under the influence of a mutagen. The virus variant was used as successful immunogen in young lambs.

Attenuated FMD live vaccines were developed by strain-passage in rabbit, chick embryos and guinea pigs. These strains were capable of inducing immunity against severe challenge with the appropriate field strain in the laboratory. However when these vaccines were used in Africa and the Middle East under different natural conditions some vaccines became pathogenic producing many outbreaks. (Mowat, Garland and Spier, 1978)

Recombinant DNA.

Vaccines from recombinant DNA for FMD virus have been developed. Since the virus has an RNA genome, complementary DNA has to be prepared first for insertion into bacterial DNA. Immunizing FMD antigen was obtained from bacteria containing the recombinant DNA (Kitching, 1992).

In 1981 Kleid, Yansura, Small, Dowbenko, Moore, Grubman, McKercher, Morgan, Robertson and Bachrach reported the successful cloning of DNA copies of segments of the FMD virus genome into bacterial plasmids. A DNA sequence coding for the immunogenic capsid protein VP3 of FMD virus A_{12} prepared from the virion RNA was legated to a plasmid designed to express a chimerical protein from the *Echerichia coli* transformed by this plasmid grown in tryptophan-depleted media. The protein obtained was inoculated into six cattle and two swine demonstrating immunogenic activity, producing neutralizing antibody and protection against FMD virus challenge.

Similar work was reported by Kupper, Keller, Kurz, Forss, Schaller, Franze, Strohmaier, Marquardt, Zaslavsky and Hofschneider, (1981) where double-stranded DNA copies of the single-stranded genomic RNA of FMD virus was cloned into *Echericha coli* plasmid pBR322. The coding sequence for structural antigen protein VP1 was identified and inserted into a plasmid vector. The viral protein VP1 synthesized in an antigenically active state in the *E. coli* cell, may provide a new way of producing a vaccine without the risk of accidental infection by incomplete inactivation of the virus.

Another similar experiment of molecular cloning of FMD virus genome was performed by Boothroyd, Highfield, Cross, Rowlands, Lowe, Brown and Harris (1981). They concluded that it was possible to develop FMD virus vaccines from genetically manipulated micro-organisms and the antigenic expression of VP1 protein in a plasmid of *E. coli* was the first step.

Elsewhere, in 1985 McKercher, Moore, Morgan, Robertson, Callis, Kleid, Shire, Yansura, Dowbenko and Small evaluated a genetically-engineered FMD virus poypeptide immunogen produced in *E. coli* was emulsified in oil adjuvant. The vaccine was used in cattle . The A_{12} VP1 fusion protein produced indicated that the biosynthetic polypeptide FMD vaccine was effective using frequent vaccination intervals followed with conventional-whole virus vaccines.

Kitching (1992) reported that the initial results of trials with DNA copies of segments of VP1 serotype "A" of FMD virus genome into bacterial plasmids infected in susceptible animal were promising in that the cloned protein produced protective antibodies. He considered that problems such as large quantities of protein required, the poor response to primary vaccination and the relative high cost of production would be resolved in due course.

6. EPIDEMIOLOGICAL APPRAISAL.

Foot-and-mouth disease is one of the most feared diseases of animals because it is very contagious and affects a wide range of animals spreading rapidly by direct and indirect means.

6.1. Endemic areas.

Spread of FMD in endemic areas is mainly by direct contact and aerosol when there is movement of infected animals to markets where they are in close contact with susceptible animals. In addition, spread of FMD occasionally may be by mechanical transmission of the virus to susceptible animals on fomites such as clothing, shoes, and veterinary instruments (Hedger and Stubbis, 1971).

Donaldson (1987) considered that the next most likely mechanism of spread is by the movement of contaminated animal products for example meat, milk, semen and skins. Of considerable epidemiological importance is the fact that infected animals excrete virus before vesicles appear, e.g. milk or semen may contain virus for up four days before disease become evident (Blackwell, 1980). FMD may also be spread on fomites, vehicles or by people. Stockmen with contaminated finger nails who "nose restrain" cattle are very likely to spread disease since cattle are very sensitive to virus entering the upper respiratory tract and associated mucosal surfaces. The excretion of virus for up of 24 hours prior to onset of clinical signs means that virus dissemination may have occurred from a farm before any suspicion of disease is raised (Donaldson, 1979).

Cottral, (1969) and Blackwell (1976-1978) commented that milk, cheese, and uncooked meat tissue, including bone, are likely to remain infected for long periods. Survival of the virus is associated with the pH of the medium and acidity in rigor mortis inactivates the virus but quick-freezing suspends acid formation and virus is likely to survive. The virus has prolonged survival probably in viscera, bone marrow, in blood vessels, and lymph nodes where acid production is not great. Likewise, Mebus and Singh (1991) reported that bovine whashed embryo from serological positive FMD animals are safe of the disease and could be imported for FMD free countries.

Rohrer in 1983 discovered that persons who handled FMD-infected animals also inhaled the virus and the agent might be isolated from naso-pharyngeal washings. Even man-to-man transfer by aerosol was observed. This interesting form of dissemination of FMDV in the respiratory tract of extremely exposed persons confirms the role of virus-containing aerosol in the direct transfer of FMD. Furthermore, the significance of these finding has still to be recognized by regulatory agencies.

Virus is most likely to initiate infection in the first animal or animals in a herd by the route by which they are most sensitive to infection; for cattle and sheep this is the respiratory tract. Routes of infection in pigs are oral and by inhalation. Once one or more animals in the herd have been infected the quantity of the virus in the environment will be greatly amplified and the transmission by several different

routes will be possible (Donaldson, 1987). Amounts of FMD virus needed to infect animals have been calculated (Seller, 1971) (Table 3).

Burrows, Mann, Garland Greig and Goodridge (1981) found that infected animals liberate large amounts of virus in secretion and excretions, before clinical signs are apparent. Virus is also excreted from infected animals in aerosol of exhaled breath and in lymph nodes from ruptured vesicles. Under suitable conditions of humidity and temperature, virus aerosols are formed and may contaminate the environment 1-10 km from their source (Sellers, 1971).

FMD virus in the environment is resistant in dried organic material. For example it was found that the virus can survive 8 to 10 weeks in hay or straw with a pH 6.7 to 9.5, at a temperature of 4°C or lower. Furthermore, FMD virus under natural conditions can survive 14 days in fecal material, 6 month in slurry in winter, 39 days in urine, 28 days on the surface of soil in autumn and 3 days on the surface of the soil in summer (Donaldson, 1987).

Thus, cattle in direct or indirect contact with the diseased will be exposed to a large amount of virus which contaminates the surface epithelia and mucosae following ingestion or inhalation of small, medium or large amounts of virus particles that are deposited in the lower and upper respiratory tracts (Terpstra, 1972).

Following the replication in the mucosae associated with the respiratory tract, virus enters the blood stream in which it may circulate for three to five days. A secondary phase of replication is initiated by blood-borne virus in organs such as lymph nodes, kidneys, mammary gland, and thyroid. The lungs and other parts of the respiratory tract are also thought to be infected during the secondary phase of virus replication in cattle during the acute phase of disease when vesicles are less than one week old. At this time, all body secretions and excretions contain high amounts of the virus. The most effective and dangerous species in regard to aerosol excretion is the pig; one infected pig can excrete $\log_{10}$ 86 ID (Infectious Doses) of FMD virus into the air (Donaldson, 1987). An example of FMD virus excretion and secretion in animals is showed in table 4.

Table 3. Doses of FMD virus needed to set infection in different species.

SPECIES	$log_{10}ID_{50}/ml$ *	ROUTE
CATTLE	2.1 - 5.1	Inhalation
SHEEP	4.0 - 8.0	Inhalation
PIG	2.6 - 6.7	Inhalation
PIG	2.6 - 5.5	Oral

* log_{10} infectious units or ineffective doses

Source: Sellers, 1971.

Table 4. FMD virus excretion or secretion in different animals.

SPECIES	SECRETION or EXCRETION	log_{10} *ID_{50}/ml
COW	MILK	6.7
COW	URINE	4.9
COW	FAECES	5.0
BULL	SEMEN	6.2
CATTLE	AEROSOL	5.1
SHEEP	AEROSOL	5.1
PIG	AEROSOL	8.6

* log_{10} infectious unites or ineffective doses.

Source: Donaldson 1987

Carriers and subclinic FMD infected animals.

Salt (1993) claimed that the carrier status and subclinic condition of FMD infected animals was characterized by asymptomatic low level excretion of FMD virus from the animal oropharynx and it depends of the FMD virus species and strains. Subclinic infected animals results when vaccination was inadequate or due to their high natural innate resistance. Likewise Seller, Hemiman and Gunn (1977) explained that transmission of FMD virus from subclinically infected vaccinated animals depends on the quantity of FMD virus in their pharynges.

Kitching (1992) described that probably subclinic infected cattle are more important in the transmission of FMD than carrier animals. Cattle will become sub clinically infected because the animal previous vaccination could have a very low infected doses or by natural resistance. The virus may be recovered from subclinically or carrier FMD infected animal by probang sampling from the pharynx.

6.2. Epidemic areas.

As is evident in the history of FMD epidemics, direct contact probably has limited significance, therefore FMD represents a typical indirectly transmitted infection in epidemic areas (Rosenberg, 1971).

Airborne dissemination.

Donaldson (1979) considered that on many occasions when FMD spread to the United Kingdom (UK) and Scandinavia it was preceded by the appearance of

outbreaks on the nearby continent. Often there was no history of contact linking outbreaks to sea-ways. Over the years, several authorities have attributed these episodes of unexplained distant spread to the carriage of the virus by wind and Barlow (1972) commented that the explosive outbreak of 1967-1968 in United Kingdom at Oswestry was attributed to airborne spread.

In 1973 Barlow and Donaldson considered that there are a large number of possible sources of air-borne virus such as directly exhaled breath of infected animals, saliva and nasal fluid. However, if the airborne virus is suspended in salivary fluid, it is less likely to survive long enough to infected a susceptible animal than if suspended nasal fluid. Donaldson (1986) in a experiment to determination the quantity and duration of FMD virus excreted by different animals reported that pigs were probably the source of airborne dissemination during outbreaks. Other possible sources included the splashing of contaminated milk, or faecal slurry; the spray disposal of infected slurry; salivary and nasal fluid, rain falling onto contaminated ground; and the burning of carcasses with vesicles. The quantity of airborne virus that these procedures potentially generate have not, however, been determined. Another possible source of airborne-virus dissemination is from bulk-milk tankers. During the filling of milk tankers, it has been observed that air displaces the milk near the air-outlet vent. The displaced air may contain droplets of FMD-infected milk. Nevertheless, airborne FMD viruses emitted by this source during outbreaks are not likely to constitute a serious hazard.

Barlow (1972) and Donaldson (1972) described that the major environmental factor on the viability of FMD virus is the relative humidity. In moist air above 55 per cent of RH, airborne virus had a low rate of inactivation but in dry air below 55 per cent RH the inactivation rate was high. Other factors which favour airborne virus dissemination are low temperature and overcast skies. Long distance spread therefore is more likely to occur in temperate rather than in tropical climates (Donaldson, Gloster, Harvey and Deans, 1982).

Donaldson and his colleagues (1982) calculated that in UK the farthest distance over which airborne spread was believed to have occurred was 250 km over the sea and 60 km over land. Likewise, Donaldson (1986) explained that once airborne virus had been emitted from a source, a plume formed which was resisted to dispersion in both horizontal and vertical planes. Prevailing climatic conditions, particularly windspeed and vertical temperature structure in the lower atmosphere, will be the major determinant of physical decay. The roughness of the surface over which the air pass influences the amount of turbulent mixing and topographical features will determiner the direction the plume travels. Virus concentration is maintained for longer over the sea than over land. These long distances are generated by continental air being vented from large buildings full of many infected pigs. The vented air is saturated with virus and plumes when vented like smoke from a factory.

In addition Rohrer (1983) commented that another form of dissemination is by birds. The virus can pass unchanged throughout the alimentary tract of bird

which may thus act as carriers and transport infection for long distances and over natural topographical barriers such as mountains ranges.

Aerobiology models.

Donaldson and his collaborators (1982) developed predictive models to forecast and analyze airborne spread during the FMD outbreak in Britain during 1981. For example, Donaldson (1986) described two mathematical models using mathematical and physical parameters; one for short-range and other for long-range prediction. The short range model is computer based and can be used to forecast the extent of dispersion of airborne virus over the land within 10 km radius of the known source. The long range model is for analyzing the dispersion of airborne virus over long distances across of the sea.

Donaldson (1994) described improved models of windborne dispersal developed by a European Community Project for computer modeling to assist in the control of epidemic exotic disease, particularly FMD. The main components were:

1. Database of agricultural and epidemic information.
2. Geographic information system (GIS) for spatial analysis and display maps.
3. Series of mathematical models to predict the production, windborne spread and likelihood of infection with FMD.

The project had other modules predicting the economic consequences of an epidemic on the spread of radioactive particles.

7. FUTURE CONTROL OF FMD IN BOLIVIA.

Foot-and-mouth disease more than any other disease has influenced the development of international codes designed to minimize the risk of introducing infection with animals into a country. Some countries have successfully avoided the introduction of disease by prohibiting the importation of all domestic animals known to be susceptible to FMD from countries where the disease exists.

For many countries that enjoy freedom from FMD such as Australia, Canada, the United Kingdom and the United States, cost-benefit analyses justify a "stamping-out" policy whenever the disease occurs or is suspect. Stamping-out is based on slaughter of affected animals and all animal exposed through direct and indirect contact, and rigid enforcement of quarantine on movement. Vaccination is not used and the policies are supported by detailed legislation and economic resources. (Mowat, 1978)

In Bolivia and in most other South American countries the FMD control programme is based on immunization with traditional inactivated vaccines in oil adjuvant and control of movement. A stamping-out policy is not possible because the prevalence of the disease in the region is believed to be too high (Rosenberg, 1971). However no protocol exist to support this belief.

7.1 Strategies for FMD control according to ecological zones.

In 1986 Astudillo and Dora considered that for the control of FMD in South America, there is a clear relationship between geographical-territorial control of FMD and the regional forms of economic and social organization of livestock farming. For example, complete cycles of breeding animals for meat production in extensive areas should have a different programme of FMD control from that used in milk production areas, or the peasant type of farming, based on trading and subsistence in areas near the cities.

The regional strategies for the control of FMD based on the type of production and ecological systems should be an important influence on control of FMD in Bolivia. The three ecological areas that are well defined in the country may require different strategies based on the systems of production in each region. In Uruguay for example, where the disease is virtually controlled in the whole country, vaccination and movement control based on ecology and the form of production of the different areas of this country permitted an effective elimination of the disease (CPFA, 1993). This system of control could be suitable for areas of milk production especially in the Bolivian valleys. In the Eastern lowlands, appropriate vaccination, identification of "carrier" herds, control of movement, and establishment of free areas could be very helpful strategies for a FMD programme. Likewise, in the highland Andean plain, control of movement and livestock markets probably are the most important factors for FMD control.

In addition Rosenberg (1986) commented that planning of FMD programs in South America at the present should consider the ecological and economic determinants of virus maintenance before its elaboration. Regional strategies in the different ecological regions, detailed legislation, political decisions and economic support are essential aspects to any successful programme.

7.2. Control by vaccination.

In many endemic countries vaccination against FMD with trivalent vaccines (containing O, A and C strains) is used, but because of increasing occurrence of antigenically dissimilar substrains, the production of vaccines from locally isolated viruses is becoming a more common practice (Donaldson, 1987).

The use of oil adjuvanted-inactivated vaccines offer promise of providing longer immunity and requiring only annual revaccination in adult cattle and twice yearly for young stock (Alonso, Casas Olascoaga, Astudillo, Sondahl, Gomez and Vianna Filho, 1987). These methods was introduced in South America where the only satisfactory strategy so far has been the vaccination (Rosenberg and Goic, 1973). Furthermore Barteling and Vreeswijk (1991) considered that double oil emulsion vaccines protect both cattle and pigs and induced long term protection. They also considered these vaccines to be most suitable for ring vaccination.

In Bolivia, inactived vaccines (O, A, C) with aluminum hydroxide, saponin or oil adjuvants are commercially available in the country but are imported from neighboring countries without adequate controls for potency and quality. These

vaccines obviously have the typical disadvantages of specificity and inappropriate potency.

Kitching (1992) considered that when vaccination is used to help control an outbreak it is necessary to choose a vaccine that contains strains antigenically similar to the outbreak strain. However as more subtypes are identified the procedure of comparing outbreak strains with each of the existing subtypes becomes cumbersome and time consuming. In addition, the results of biochemical analysis of FMD virus make it apparent that this one-dimensional classification of FMD virus is no longer sustainable (Kitching et al, 1989).

In the WRL outbreak strains are now compared directly with existing vaccines strains using serum derived from bovines 21 days after of vaccination. A rapid antigenic comparison can be carried out by ELISA and that allows the selection of the most suitable available vaccine strains (Samuel, Knowles and Kitching, 1991).

Furthermore in the International Vaccine Bank at Pirbright different vaccines from different parts of the world have been stored and offer a wide diversity of coverage in the field. The high potency of different strains such as O_1 Lausance, A_{24} Cruzeiro, A_{22} Iraq 24/26, C_1Oberbayen and Asia India 8/9 provide a broader range of isolates than that normally observed. However the constantly changing epidemiological situation worldwide requires continued monitoring of the viruses that are prevalent at any given time.

For a programme of vaccination in Bolivia it is important that monitoring and identification of the virus be continued. Serological testing for the presence of antibodies are very useful for screening animals prior to movement; for vaccine potency testing, for monitoring the extent and effectiveness of vaccination in the field and for epidemiological studies of diseases in animal population (Samuel, Knowles, Samuel and Crowther, 1991).

Another factor that is an important consideration in vaccination programmes is that inapparent infection may occur in animals whose resistance induced by vaccination has waned permitting the existence of "carrier" status (Donaldson and Kitching, 1989). It has become recognized that the number of carrier animals produced by vaccination is very much greater than previously thought. Apart from the fact that these animals are a potent method of spreading the disease and they also provide an excellent medium for mutation of existing virus strains, because the hosts are immune (Salt, 1993).

In the future the use of synthetic vaccines may be relevant in the control of FMD worldwide but much work still needs to be done and the new vaccines cannot yet replace the classical inactivated vaccines (Barteling and Vreeswijk, 1991).

7.3. No vaccination.

Mowat (1978) explained that vaccination with live inactivated and attenuated vaccines had been used with some success in Africa and in some

countries in South America but are not sufficiently stable in their properties to be considered for use in European countries or in meat-exporting countries.

In South America near to 1000 million doses are produced commercially in the endemic countries but many of these vaccines does not have adequate quality controls (Della Porta, 1983). Obviously, production of FMD vaccines is good business and there are too many laboratories in different countries with a vestal interest in producing FMD vaccines.

In 1987 Beck and Strohmaier considered that inactivated vaccines, especially formalin-inactivated ones, were the origin of many outbreaks in different parts of the world. and they suggested that it is important to impose stricter controls in the handling of the virus in vaccine production plants and in laboratories working with viable virus. Amadozi, Archetti, Tollis, Buonovaglia and Panina (1991) explained that the use of vaccines for FMD control or eradication demand considerable organization and financial efforts.

European countries, by stopping the use of vaccine and applying a strict stamping out policy, reached effective control of the disease. However, the European Economic Community (EEC) retains the option to employ emergency vaccination should there be an outbreak of FMD which cannot be immediately controlled by slaughter (Kitching, 1992).

In Bolivia the use of stamping out policy, could be an alternative to the present method used for control and eradication of the disease. However many economic, social and biological aspects must be considered. Cost-benefit analysis, surveillance, biotechnology and control of animal movement should be carrying out before any determination or decision.

7.4. Surveillance and animal movement.

Rosenberg and Goic (1973) argued that in South American countries the establishment of important prophylactic measures is important to avoid the dissemination of FMD. Control of animal movement, quarantine of affected farms, disinfecting of vehicles, materials and equipment are all important for effective FMD control. Legislation in South American countries including Bolivia recognize this situation, but the application of legislation is not always possible because in extensive areas there are no systems for the monitoring of animal movements. However in Chile and Uruguay control of the animal movement and surveillance have permitted a successful control of FMD.

Furthermore in Bolivia, surveillance of animal movement on the borders of the country are essential for FMD control. Coordination between the Bolivian veterinary services and their neighbors could diminish the incidence of FMD in the country.

In addition, Forman (1990) commented that movement control alone would be of greater benefit than dependence on vaccination for the control. He gave as an

example Thailand, where careful control of animal movement into the Southern peninsula has enabled the region to remain free of the disease for long periods.

7.5. Biotechnology.

Kitching (1992) postulated that the potential use of biotechnology is an epidemiologic alternative for the control of FMD. The identification and characterization of FMD strains, the development of new molecular FMD vaccines, the mechanism of dissemination of the disease, surveillance, and other new techniques are unquestionably important for the control of the disease (Mebus and Singh, 1991).

In many countries in which FMD still occurs, the prospect of exporting meat to FMD-free countries has encouraged efforts to control and eradicate the disease. As the level of surveillance rises it becomes more evident that the carrier animal is extremely important in the epidemiology of FMD but the lack of knowledge in the identification of the carrier and unsolved immunological problems with new vaccines are factors that the rapid progress in biotechnology may be able to answer. These answers will be useful to the elimination of FMD particularly in developing countries as Bolivia.

8. CONCLUSION.

FMD is the most infectious disease of animals. A prime consideration is the speed with which the disease spreads through animal populations, producing severe economic losses. Consequently, in recent years, research in FMD has been well supported and has identified many new features.

The new discoveries in biotechnology have been utilized in FMD studies as a new alternative for the control of the disease. For example, in diagnosis, the standardization of ELISA has replaced complement fixation test and virus neutralization test for FMD antigen detection and serology. Likewise monoclonal antibodies have been used for detection of the antigenicity of the virus and for many research purposes. Furthermore in the future the utilization of sophisticated diagnosis tests with collect precise data on disease incidence and spread-status allowing the elaboration of predictive models for FMD control.

In epidemiology the most important new factors identified are the "carrier" and "subclinical" state of vaccinated and not vaccinated animals. Different asymptomatic species are carriers of FMD virus in their pharyngeal region. In epidemic areas airborne dissemination has been intensely studied to avoid the penetration of the disease.

For FMD control there are some new approaches. The association of ecological, economic and social systems of production in relation to the presence of

FMD virus presence are considered as determinants for FMD control in South American countries. The technique could be applied in Bolivia where the three characteristic ecological regions have their own peculiarities of production.

In vaccination, considering that FMD virus occurs in seven immunologically different serotypes which are divided into a number of subtype strains, identification of the antigenic similarities of virus strains should be a compulsory requirement for vaccine selection. In Pirbright (UK) the International Vaccine Bank has been established where the different strains of virus that are used in vaccine production in different parts of the world are coded and stored.

Likewise, due to different problems of traditional vaccines a new generation of vaccines based mainly on synthetic peptides and DNA recombinants have been studied as a new option for FMD control. Nonetheless for a successful vaccine immunological, antigenic and economic considerations need to be resolved.

FMD in Bolivia is endemic and is partially uncontrolled. It causes severe losses in livestock productivity, reduces the opportunity for export animal products and causes destructive effects to the economy of the rural population. Therefore the establishment of a national programme for FMD control in the different regions is imperative in a country where the livestock is the livelihood of many people.

The new FMD features are certainly tools that could be used in the control of the disease in Bolivia. Nevertheless reorganization of the animal health

programmes and a decisive government support should be the initial basis to the control in the country.

The utilization of non-biological strategies such as adequate legislation, political decision, associated with bio-technological strategies including surveillance, monitoring, quarantines, vaccination, and new diagnostic tests will be important elements for a successful future FMD control in Bolivia.

Finally, FMD in Bolivia is a disease that can be controlled or even eradicated and the successes of these objectives will unquestionably benefit the rural human population and the national economy. However it will be possible only if there is good motivation and co-operation among veterinarians, governmental institutions and livestock owners.

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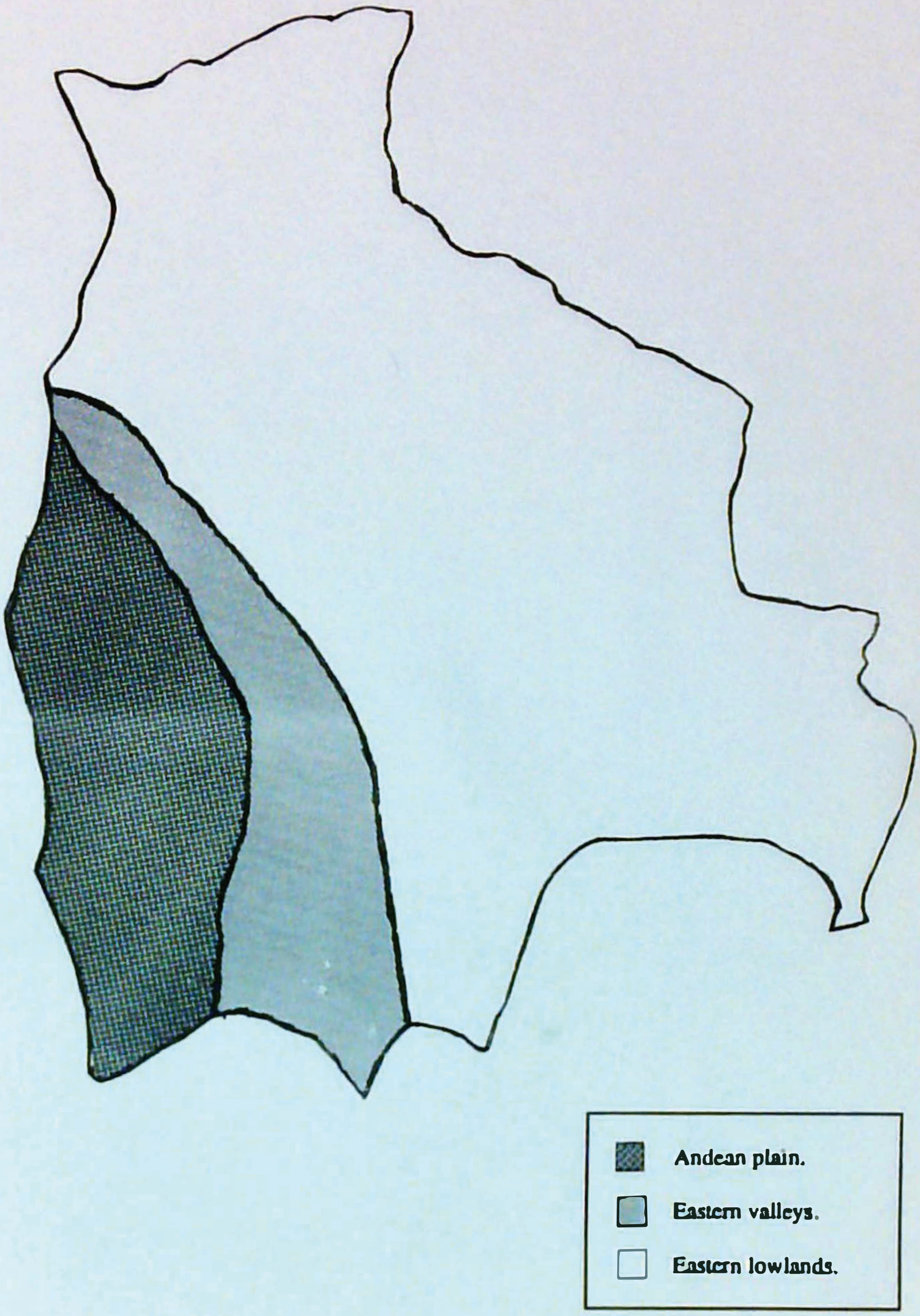
Appendix 1. Ecological map of Bolivia.



Source: I.N.E., 1993.